(5)(i) Evaluated on the basis of information submitted as part of the application and any other information before the Food and Drug Administration with respect to such drug, there is lack of substantial evidence consisting of adequate and well-controlled investigations, including clinical (field) investigation, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and reasonably be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling.

(ii) The following principles have been developed over a period of years and are recognized by the scientific community as the essentials of adequate and well-controlled clinical (field) investigations. They provide the basis for the determination whether there is *substantial evidence* to support the claims of effectiveness for *new animal drugs.*

(*a*) The plan or protocol for the study and the report of the results of the effectiveness study must include the following:

(*1*) A clear statement of the objectives of the study.

(*2*) A method of selection of the subjects that—

(*i*) Provides adequate assurance that they are suitable for the purposes of the study, diagnostic criteria of the condition to be treated or diagnosed, confirmatory laboratory tests where appropriate, and, in the case of prophylactic agents, evidence of susceptibility and exposure to the condition against which prophylaxis is desired;

(*ii*) Assigns the subjects to test groups in such a way as to minimize bias; and

(*iii*) Assures comparability in test and control groups of pertinent variables, such as species, age, sex, duration and severity of disease, management practices, and use of drugs other than those being studied. When the effect of such variables is accounted for by an appropriate design, and when, within the same animal, effects due to the test drug can be obtained free of the effects of such variables, the same animal may be used for both the test drug and the control using the controls set forth in paragraph (a)(5)(ii)(*a*)(*4*)(*i*), (*ii*), or (*iii*) of this section.

(*3*) An explanation of the methods of observation and recording of the animal response variable studied and the means of excluding bias or minimizing bias in the observations.

(*4*) A comparison of the results of treatment or diagnosis with a control in such a fashion as to permit quantitative evaluation. The precise nature of the control must be stated and an explanation given of the methods used to minimize bias on the part of the observers and the analysts of the data. Level and methods of ''blinding,'' if used, are to be documented. Generally, four types of comparisons are recognized:

(*i*) No treatment: Where objective measurements of effectiveness are available and placebo effect is negligible, comparison of the objective results in comparable groups of treated and untreated animals.

(*ii*) Placebo control: Comparison of the results of use of the new animal drug entity with an inactive preparation designed to resemble the test drug as far as possible.

(*iii*) Active treatment control: An effective regimen of therapy may be used for comparison, e.g., where the condition treated is such that no treatment or administration of a placebo would be contrary to the well-being of the animals.

(*iv*) Historical control: In some circumstances involving diseases with high and predictable mortality (leukemia or tetanus) or with signs and symptoms of predictable duration or severity (some forms of parasitism, bovine hypocalcemia, canine eclampsia) or in the case of prophylaxis where morbidity is predictable, the results of use of a new animal drug entity may be compared quantitatively with prior experience historically derived from the adequately documented natural history of the disease or condition in comparable animals with no treatments or with a regimen (therapeutic, diagnostic, prophylactic) whose effectiveness is established.